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HEMATOLOGIC CHARACTERISTICS OF THE WOYLIE (*BETTONGIA PENICILLATA OGILBYI*)

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ABSTRACT: An accurate assessment of animal health is fundamental to disease investigation in wildlife. Blood samples ($n=609$) from several populations of the endangered woylie or brush-tailed bettong (*Bettongia penicillata ogilbyi*), collected between March 2006 and April 2010 in Western Australia and South Australia, were used to establish hematologic reference ranges. Differences between populations, sexes, and seasons were also investigated. Significant sex differences in hematocrit, red blood cell, total white blood cell, neutrophil, lymphocyte, and eosinophil counts were evident in at least one population. Generally, males had higher hematocrit and blood cell concentrations than did females. A positive association of the erythron parameters with rainfall was also detected. The hematologic characteristics of woylie populations described in this study greatly increase knowledge of the health status in these populations. The data also represent a baseline to enable monitoring and detection of changes in the health status in these populations as well as representing a valid dataset for comparison with hematologic investigations in other macropods and marsupials.

Key words: Bettong, health assessment, hematology, macropods, seasonality, wild populations.

INTRODUCTION

Health assessment and disease investigations are dependent on accurate detection of variation in health status. With wildlife this can be challenging because it may be difficult to establish reference ranges for physiologic parameters and define what can be considered "normal" (Wobeser, 2007). While emerging infectious diseases associated with high mortality rates are of great concern (Daszak and Cunningham, 1999), clinical conditions that result in chronic diseases but low fatality rates, or that have sublethal effects, can also profoundly affect populations (Spalding and Forrester, 1993). Sensitive diagnostic tools are especially important to detect diseases that are carried subclinically by individuals. The importance of such tools is further highlighted by the difficulty in sampling wild animals throughout all stages of a pathologic process. This is because sampling may be biased toward healthier animals, as affected animals become increasingly rare or undetectable with the advance of the disease. Therefore, linking what might seem an unremarkable finding to a preliminary stage of a serious clinical condition can be critical to successful diagnosis.

In several wildlife studies hematologic changes, which were the only detectable abnormality in the study, were associated with reduced survival despite the inability to identify clearly the underlining pathologic process (e.g., Mathews et al., 2006). Hematology is believed to be a reliable indicator of general health in many mammals (Coles, 1986; Kerr, 2002; Clark, 2004). This is also true for macropods, despite the limited research on hematologic responses in these species and the fact that animals with severe clinical signs might not show hematologic changes of the magnitude expected in eutherian mammals (Vogelnest and Portas, 2008).

We describe the hematologic characteristics of the woylie (*Bettongia penicillata ogilbyi*), an Australian macropod that has recently undergone a dramatic population decline (approximately 90% between 1999

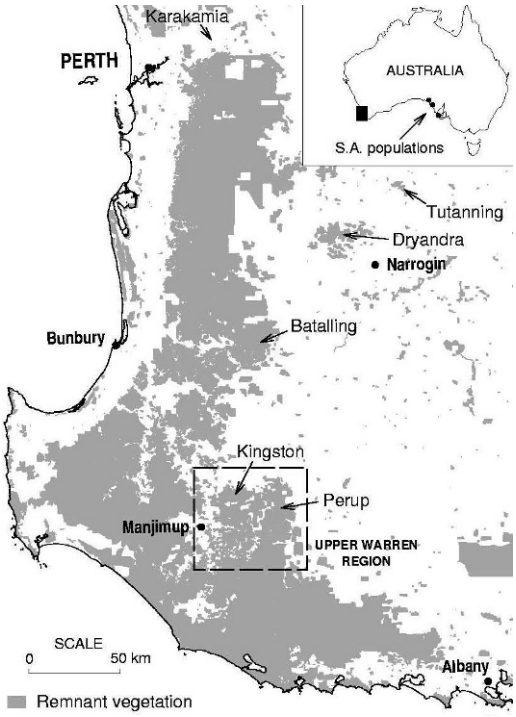

FIGURE 1. Geographic location of sampled woylie (*Bettongia penicillata ogilbyi*) populations in Western Australia and South Australia (S. A.; inset). Modified with permission from Pacioni et al., 2011.

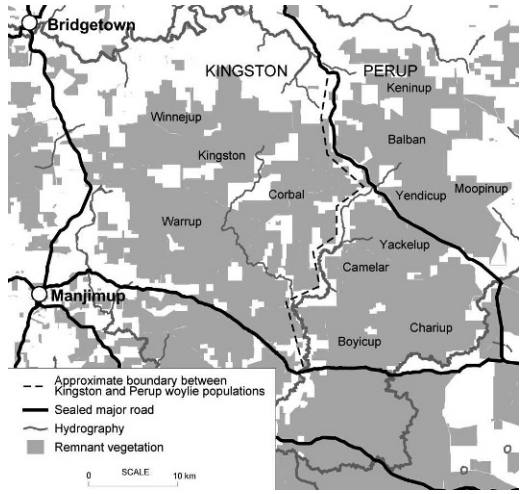

FIGURE 2. Forest blocks sampled within the Upper Warren region, Western Australia (Reproduced with permission from Pacioni et al., 2011).

and 2010; Wayne et al., 2009, 2013; Groom, 2010). Based on the available evidence, it was hypothesized that predators or disease may be the primary causes of the decline (Wayne, 2006). Therefore, it was particularly important to detect changes in the health status of individuals from monitored populations. We establish reliable hematologic reference ranges, investigate differences in hematologic parameters between populations and sexes, and evaluate the influence of climatic conditions on woylie hematology. These findings are discussed and compared with currently available hematologic information for other marsupials.

MATERIALS AND METHODS

We trapped 1,248 woylies using standard protocols (Department of Environment and Conservation [DEC] Science Division, 2008) in several indigenous and translocated populations in Western Australia and South Australia (Fig. 1). The two genetically distinct populations (Perup and Kingston; 34°10′S, 116°35′E and 34°08′S, 116°21′E, respectively; Fig. 2) within the contiguous jarrah (*Eucalyptus marginata*) forest of the Upper Warren region (Pacioni et al., 2011) were sampled intensively between March 2006 and April 2010. The population in Karakamia Wildlife Sanctuary (Karakamia, 31°49′S, 116°15′E) was sampled in July 2006 and November 2007. The populations in Dryandra woodland (Dryandra, 32°47′S, 116°55′E), Tutanning Nature Reserve (Tutanning, 32°31′S, 117°18′E), and Batalling State Forest (Batalling, 33°29′S, 116°32′E) were sampled in November 2006. In South Australia, St. Peter Island (SPI, 32°16′S, 133°35′E) and Venus Bay Island (33°12′S, 134°40′E) populations were sampled in June 2007. After a population crash in Venus Bay Conservation Park (Venus Bay CP, 33°13′S, 134°40′E), woylies were moved temporarily to Monarto Zoo (Adelaide) and blood samples were taken shortly after this move in December 2006. Individuals were identified by ear tags or microchips. Weight, head length, sex, reproductive status (including presence of pouch young), age (juvenile, subadult, or adult), and body and coat condition (as a score from 1 to 5) were also recorded. The complete dataset was not always available for individuals captured in populations other than Perup and Kingston.

TABLE 1. Sample size (*n*), mean, standard deviation (SD), and 5th and 95th percentiles of hematologic parameters in woylie (*Bettongia penicillata ogilbyi*) populations.

	RBC[a] ($\times 10^{12}$/L)	HGB[a] (g/L)	HCT[a] (L/L)	MCV[a] (fL)	MCH[a] (pg)	CHCM[a] (g/L)
Perup						
n	104	104	104	104	104	103
Mean (SD)	11.66 (1.09)	161.38 (12.68)	0.53 (0.05)	45.35 (3.05)	13.89 (0.95)	302.69 (16.18)
5th–95th Perc[b]	9.66–13.34	138–181	0.44–0.6	40.63–51.3	12.43–15.53	268.4–326.6
Kingston						
n	43	43	43	43	43	43
Mean (SD)	10.15 (1.06)	150.09 (16.17)	0.49 (0.05)	48.25 (3.43)	14.87 (1.55)	302.51 (13.54)
5th–95th Perc	8.46–12.51	116.2–175.8	0.41–0.57	41.3–54.66	11.2–17.26	274.2–324.8
Venus Bay CP						
n	32	32	32	32	32	32
Mean (SD)	11.29 (1.04)	171.5 (13.17)	0.55 (0.05)	48.36 (3.41)	15.24 (0.96)	333.81 (15.14)
5th–95th Perc	8.86–12.6	139.75–191.05	0.43–0.61	43.16–54.97	13.63–17.01	305.55–363.85
St. Peter Island						
n	42	42	42	42	42	42
Mean (SD)	8.75 (1.39)	129.05 (23.88)	0.41 (0.06)	47.43 (3.84)	14.85 (2.24)	306.38 (14.35)
5th–95th Perc	5.72–11.1	75.15–161.4	0.27–0.51	39.89–52.74	10–18.2	278–329.1
Overall						
n	270	270	270	270	270	269
Mean (SD)	10.76 (1.52)	155.4 (20.41)	0.5 (0.06)	46.75 (3.57)	14.53 (1.46)	308.06 (17.26)
Mean (SD) Btst[c]	10.76 (1.52)	155.32 (20.4)	0.5 (0.06)	46.75 (3.56)	14.53 (1.46)	308.05 (17.21)
5th–95th Perc	8.27–12.96	117.1–183	0.39–0.59	41.06–52.98	12.2–16.95	278–338.5
5th–95th Perc Btst	8.2–12.95	117.28–182.73	0.39–0.59	41.06–52.95	12.25–16.95	278.78–338.13

[a] RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; MCV = mean cell volume; MCH = mean corpuscular hemoglobin; CHCM = corpuscular hemoglobin concentration mean; WBC = total white blood cells; Neutro = neutrophils; Lympho = lymphocytes; Mono = monocytes; Baso = basophils; TP = total protein concentration; FBG = fibrinogen concentration.

[b] Perc = percentiles.

[c] Btst = bootstrapped.

Blood samples were collected from the lateral tail vein of a subset of individuals (Table 1), mixed with ethylenediaminetetraacetic acid in commercial tubes, and chilled on wet ice in the field. Blood smears were made at the time of collection and air dried. In Upper Warren, animals were sampled immediately after removing the animals from the traps early in the morning. In South Australia, animals were processed throughout the night while in Karakamia, animals were removed from traps during the night and kept in bags until early morning when blood samples were collected.

Blood samples were processed at Murdoch University Clinical Pathology Laboratory within 36 hr of collection as recommended for macropods (Hulme-Moir et al., 2006). Differential white blood cell (WBC) counts were carried out manually by examination of blood smears using light microscopy while other parameters (Table 1) were measured with an automatic hematology analyzer (ADVIA-120) using multispecies software (Bayer diagnostics division, Tarrytown, New York, USA). Platelet count and mean platelet volume were not included in analyses because aggregates of platelets were frequent on blood smears. Packed cell volume (after centrifugation of a capillary tube) and mean corpuscular hemoglobin concentration (MCHC) were plotted and graphically checked for consistency with hematocrit (HCT; calculated from red blood cell [RBC] count and mean cell volume [MCV]) and corpuscular hemoglobin concentration mean (CHCM; an MCHC direct measurement determined by flowcytometric signal; Bosch et al., 1992), respectively. When discordant, blood samples were removed from the dataset (Bosch et al., 1992) as were samples that showed macroscopic signs of hemolysis. Total protein (TP) concentration

TABLE 1. Extended.

WBC[a] ($\times 10^9$/L)	Neutro[a] ($\times 10^9$/L)	Lympho[a] ($\times 10^9$/L)	Mono[a] ($\times 10^9$/L)	Eos[a] ($\times 10^9$/L)	Baso[a] ($\times 10^9$/L)	TP[a] (g/L)	FBG[a] (g/L)
104	104	104	104	101	104	98	21
5.65 (2)	2.13 (0.94)	3 (1.68)	0.13 (0.11)	0.17 (0.21)	0.02 (0.03)	66.74 (4.01)	2.19 (0.6)
2.7–10.03	0.83–3.91	0.64–6.24	0–0.37	0–0.53	0–0.09	59–74	1–3
43	43	43	43	43	43	42	15
5.23 (2.11)	1.86 (1)	2.69 (1.28)	0.14 (0.14)	0.34 (0.45)	0.03 (0.04)	64.98 (5.35)	2.07 (0.59)
2.54–9.88	0.48–4.05	0.82–5.15	0–0.48	0–0.95	0–0.12	56.3–77.95	1–3
32	32	32	32	32	32	32	—
5.57 (2.29)	3.2 (2.11)	1.95 (1.46)	0.17 (0.13)	0.09 (0.12)	0.03 (0.05)	71.97 (8.88)	—
2.29–10.66	0.94–8.08	0.54–5.56	0.04–0.43	0–0.43	0–0.18	51.5–86.7	—
42	42	42	42	42	42	42	42
4.05 (2.31)	2.61 (1.6)	1.25 (1.09)	0.13 (0.15)	0.05 (0.07)	0.01 (0.02)	66.12 (3.73)	2.12 (1.02)
1.02–9.2	0.4–5.46	0.29–4.42	0–0.42	0–0.23	0–0.09	61–74.85	1–4
270	270	270	270	267	270	262	98
5.23 (2.16)	2.49 (1.52)	2.31 (1.57)	0.13 (0.12)	0.15 (0.25)	0.02 (0.03)	66.86 (5.52)	2.09 (0.81)
5.23 (2.16)	2.49 (1.51)	2.31 (1.56)	0.13 (0.12)	0.15 (0.24)	0.02 (0.03)	66.83 (5.49)	2.09 (0.81)
1.96–9.8	0.69–5.37	0.51–5.23	0–0.39	0–0.52	0–0.09	59–75.85	1–4
2.02–9.56	0.71–5.4	0.5–5.27	0–0.39	0–0.53	0–0.09	58.98–75.98	1–3.58

was assessed by refractometry and fibrinogen (FBG) concentration by heat precipitation (Coles, 1986).

Although the ratio of neutrophils to lymphocytes (N:L) has been used as an indicator of health in some marsupial studies (e.g., Presidente and Correa, 1981), it was not used here because of variability within individuals and inconsistent results, especially in macropods (McKenzie et al., 2002; Clark, 2004; Young and Deane, 2006).

Generally, 2.5th and 97.5th percentiles are used to establish normal hematologic reference ranges (Lumsden and Mullen, 1978; Solberg, 1987; National Committee for Clinical Laboratory Standards, 1995); however, a more conservative approach was used due to the lack of clinical history of individuals and the relatively small sample size in some populations. Reference ranges for the adult age class (only) were established as the 5th and 95th percentiles of each parameter distribution after removing outliers (Lumsden and Mullen, 1978). Additional criteria were used for inclusion of animals trapped within the Upper Warren region to calculate the reference ranges: valid morphologic measurements, an average body and coat condition score equal to or higher than 3.5; a biometric index above the 5th percentile (see below); and lastly, animals had to be alive (i.e., retrapped) at the following trapping session.

The biometric index was calculated as the ratio of the weight:head length. The minimum threshold to consider a woylie in "good" condition was established by calculating the 5th percentile, after controlling for sex, and removing outliers and females with pouch young. Biometric indices were analyzed only for woylies trapped at Kingston, Perup, and Karakamia because morphometric data were insufficient from other populations.

When there were statistical differences between populations or sexes, these groups were considered separately. Only individuals that had blood parameters within the established reference ranges were retained for further analyses.

Statistical analysis

All statistical analyses were carried out in SPSS version 19 (SPSS Inc., Chicago, Illinois, USA) and when multiple samples for the same individual were present, one was randomly

selected for inclusion in the analysis to comply with the assumption of independence. The distributions of variables were plotted and inspected, and the Kolmogorov-Smirnov test and Z-test were used to confirm a normal distribution following Field's (2009) guidelines. Variables that were not normally distributed were transformed (square root). The homogeneity of variance was tested with the Levene's test.

Lack of compliance with the assumptions for the multivariate analysis of variance prevented the use of this statistical approach (Stevens, 2002). Therefore, two-way analysis of variances (ANOVAs) were used to compare the mean differences between populations and genders for RBC, hemoglobin (HGB), HCT, TP, WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts. Hochberg's GT2 post hoc tests were used to follow up differences between groups, and parametric *t*-tests were used to explore differences between sexes within the same populations. When the assumption of homogeneity of variances was not met, multiple one-way ANOVAs were used and Welch's robust tests were considered when the variances were still unequal between groups. In these cases, Games-Howell post hoc tests were used (Field, 2009). The nonparametric Mann-Whitney *U*-test or Kruskal-Wallis test were used to compare two or more groups respectively when variables did not have a normal distribution and to investigate differences in rainfall within the same seasons between years and the two Upper Warren populations.

Seasonal changes could not be directly investigated because fewer samples were obtained in winter and summer. Nevertheless, associations between selected hematologic parameters and rainfall (Bureau of Meteorology, 2010) were considered. These analyses were limited to animals trapped within 15 km of the weather stations within Perup and Kingston (weather stations 9616 and 9906, respectively). Initially, Spearman's rho coefficients (r_s) were calculated using two rainfall variables: monthly rainfall and the average rainfall for the 2-mo period including the sampling month and the month prior to sampling. Only the variable that showed the strongest correlation with the hematologic parameters was used in further analyses (see below). Finally, rainfall, "period of the year" (cold or warm), and population of origin were entered one at a time in a hierarchical multiple regression model. In doing so, the effect of each variable on the hematologic parameters was calculated and partialled out. Significant improvement of the model was evaluated with an *F*-ratio test. Only the final models are reported (i.e., including only variables that made a significant contribution). Standard guidelines were followed to verify compliance with assumptions (Durbin and Watson, 1951; Myers, 1990; Menard, 1995; Tabachnick and Fidell, 2007).

Statistical significance was set to 0.05. However, control of type I error (rejecting the null hypothesis when it is true) was achieved with the Benjamini-Hochberg approach (Benjamini and Hochberg, 1995). This approach was applied only when the statistical tests involved analysis of the same variable in different groups.

RESULTS

Influence of populations and sex

All hematologic parameters were normally distributed except for monocyte, eosinophil, and basophil concentrations within each population and sex, and excepting lymphocyte counts for males in Venus Bay CP and females in SPI. The square root transformations of monocyte and eosinophil concentrations were normally distributed and used for statistical analyses. After removal of hemolytic samples and outliers, sample sizes for Karakamia, Dryandra, Tutanning, Batalling, and Venus Bay Island were very small ($n<20$), and data from these populations were not included in any statistical analysis except for the calculation of "overall species" reference ranges (see below).

All the considered hematologic parameters were significantly different between populations (Table 2) except for monocyte concentrations. Within the erythron panel, post hoc analyses revealed that each population differed significantly from any other population except for Perup and Venus Bay CP, where only HGB differed significantly (Table 3). Within the leukocyte panel, South Australia populations showed a significant difference from each other only in the lymphocyte concentration, while Kingston and Perup were not different between each other and, instead, differed from Venus Bay CP and SPI in most parameters (Table 3).

TABLE 2. Statistical significance (*P*-values) of hematologic differences between sexes and populations of the woylie (*Bettongia penicillata ogilbyi*). Significant *P*-values are indicated in boldface (after Benjamini-Hochberg correction [Benjamini and Hochberg, 1995]).

Parameter[a]	Perup[b]	Kingston[b]	Venus Bay CP[b]	SPI[b]	Between populations	Between genders	Males between populations	Females between populations
RBC	>0.05	**0.009**	>0.05	>0.05	**<0.0005**	**0.018**	**<0.0005**	**<0.0005**[c]
HGB	>0.05	>0.05	>0.05	>0.05	**<0.0005**	>0.05	**<0.0005**	**<0.0005**[c]
HCT	>0.05	0.024	>0.05	>0.05	**<0.0005**	>0.05	**<0.0005**	**<0.0005**[c]
TP	>0.05	>0.05	>0.05	>0.05	**0.002**[c]	>0.05	>0.05[c]	**<0.0005**
WBC	>0.05	**0.002**	>0.05	>0.05	**0.001**[d]	**0.007**[d]	0.045	**0.008**
Neutrophils	>0.05	>0.05	0.019	>0.05	**0.004**[c]	**0.018**	**0.005**[c]	>0.05[c]
Lymphocytes	>0.05	**0.012**	0.006[e]	>0.05[e]	**<0.0005**[f]	>0.05	**<0.0005**[f]	**<0.0005**[f]
Monocytes[g]	>0.05	0.018	>0.05	>0.05	>0.05[d]	>0.05[d]	>0.05	>0.05
Eosinophils[g]	0.039	>0.05	>0.05	>0.05	**<0.0005**[c]	>0.05	**<0.0005**[c]	**<0.001**[c]

[a] RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; TP = total protein concentration; WBC = total white blood cells; CP = Conservation Park; SPI = St. Peter Island.

[b] Comparisons between sexes within population.

[c] Welch's robust tests of equality of means.

[d] Two-way analysis of variance.

[e] Mann-Whitney *U*-test.

[f] Kruskal-Wallis test.

[g] Square root of raw data.

Several parameters showed a significant difference between sexes (Table 2), although the comparisons within populations did not always remain significant after Benjamini-Hochberg correction. Kingston was the only population where more than one hematologic parameter was significantly different between sexes (Table 2), with males having higher cell counts (Table 4). This trend was also present in the other populations with the exception of Venus Bay CP (Tables 3 and 5). Due to these statistical differences, reference ranges were calculated separately for each population. Moreover, when at least one parameter was significantly different between males and females, the 5th and 95th percentiles were reported separately (Table 1 and 5).

Reference ranges were also calculated by combining all available data along with 1,000 bootstrapped estimates as suggested for hematologic reference ranges when $n>100$ (Table 1; Linnet, 2000). These should represent the likely overall species ranges for blood parameters. This was done to provide indicative ranges by which populations not studied here might be generally compared.

Climatic influence

The monthly rainfall in Upper Warren ranged from 0 to 162 mm (Fig. 3) with summer months being the driest, as expected. No significant difference was found for the same season between years and between the two populations in the Upper Warren region. The monthly rainfall had significant correlations with all hematologic parameters except TP, neutrophil, monocyte, and eosinophil concentrations. This variable was then used in the hierarchical multiple regression analyses and, from here on, is referred to as "rainfall."

There was a significant positive association between rainfall and RBC counts, hemoglobin concentration, and HCT. The population of origin still had a significant effect despite controlling for the rainfall effect (Table 5). The WBC and lymphocyte counts were negatively associated with rainfall while neutrophil and eosino-

TABLE 3. Statistical significance (*P*-value) of post hoc tests of hematologic parameters between woylie (*Bettongia penicillata ogilbyi*) populations. Post hoc tests for monocyte concentrations are not reported because a nonsignificant difference was found between populations in the univariate analysis.[a]

Population	Parameter	Group	Perup	Kingston	Venus Bay CP	SPI
Perup	RBC	overall	—[b]	<0.0005	0.510	<0.0005
		males	—	<0.0005	0.334	<0.0005
		females	—	<0.0005	0.976	<0.0005
	HGB	overall	—	0.001	0.002	<0.0005
		males	—	0.021	0.155	<0.0005
		females	—	0.033	0.013	<0.0005
	HCT	overall	—	<0.0005	0.381	<0.0005
		males	—	0.018	0.806	<0.0005
		females	—	0.001	0.407	<0.0005
	TP	overall	—	0.229	0.014	0.810
		females	—	0.114	0.007	0.973
	WBC	overall	—	0.850	1.000	<0.0005
		females	—	0.187	1.000	0.011
	Neutrophils	overall	—	0.441	0.041	0.276
		males	—	0.884	0.017	0.213
		females	—	0.171	0.990	0.799
	Lymphocytes	overall	—	0.350	0.001	<0.0005
		males	—	0.960	<0.0005	<0.0005
		females	—	0.134	0.563	<0.0005
	Eosinophils[c]	overall	—	0.078	0.051	<0.0005
		males	—	0.360	<0.0005	<0.0005
		females	—	0.107	0.586	0.208
Kingston	RBC	overall		—	<0.0005	<0.0005
		males		—	0.049	<0.0005
		females		—	<0.0005	0.040
	HGB	overall		—	<0.0005	<0.0005
		males		—	0.001	0.004
		females		—	<0.0005	0.030
	HCT	overall		—	<0.0005	<0.0005
		males		—	0.007	<0.0005
		females		—	0.001	0.035
	TP	overall		—	0.001	0.669
		females		—	0.007	0.973
	WBC	overall		—	0.981	0.060
		females		—	0.590	0.988
	Neutrophils	overall		—	0.010	0.058
		males		—	0.100	0.213
		females		—	0.539	0.197
	Lymphocytes	overall		—	0.004	<0.0005
		males		—	<0.0005	<0.0005
		females		—	0.771	0.001
	Eosinophils[c]	overall		—	0.001	<0.0005
		males		—	<0.0005	<0.0005
		females		—	0.981	0.002
Venus Bay CP	RBC	overall			—	<0.0005
		males			—	<0.0005
		females			—	<0.0005
	HGB	overall			—	<0.0005
		males			—	<0.0005
		females			—	<0.0005
	HCT	overall			—	<0.0005
		males			—	<0.0005
		females			—	<0.0005
	TP	overall			—	0.006

TABLE 3. Continued.

Population	Parameter	Group	Perup	Kingston	Venus Bay CP	SPI
		females			—	0.008
	WBC	overall			—	0.014
		females			—	0.162
	Neutrophils	overall			—	0.559
		males			—	0.270
		females			—	0.976
	Lymphocytes	overall			—	0.005
		males			—	0.255
		females			—	<0.0005
	Eosinophils[c]	overall			—	0.282
		males			—	0.999
		females			—	0.010

[a] CP = Conservation Park; SPI = St. Peter Island; RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; TP = total protein concentration; WBC = total white blood cells.

[b] (—) = not applicable.

[c] The square root of raw variable (for eosinophils) was used in the analysis.

phil concentrations were associated only with the period of the year (Table 5). Lastly, TP was also (and uniquely) associated with the period of the year (Table 5).

DISCUSSION

Influence of populations and gender

The reference ranges we calculated had a higher concentration of RBC accompanied by lower MCV, mean corpuscular hemoglobin (MCH), and CHCM than did the reference ranges of other macropods (Clark, 2004; see Vogelnest and Portas, 2008 for a review). These findings are also consistent with preliminary woylie hematologic investigations (Clark, 2008). Nevertheless, the leukocyte panel is broadly similar to other macropods (Clark, 2004; Vogelnest and Portas, 2008). In many marsupials, lymphocytes are the most abundant leukocyte in the peripheral blood (Clark, 2004) but this is not necessarily the case in macropods (Vogelnest and Portas, 2008). Lymphocyte concentrations were significantly different between woylie populations. In most populations, neutrophils were evidently the most common WBC type while in the Upper Warren populations counts of lymphocytes and neutrophils were similar.

It is not clear whether the differences between woylie populations in the leukocyte panel are due to physiologic differences, sampling biases, or different immune system stimulations. Fear and pain can increase neutrophil and lymphocyte concentrations in marsupials (Clark, 2004), and the differences in trapping and handling protocols could be responsible for these discrepancies. Similarly, environmental fluctuations or captivity (animals from Venus Bay CP) may partially account for these differences. Nevertheless, the two Upper Warren populations were sampled during their decline or shortly after, while the other wild populations (with >10 samples) were demographically stable. The association of the immune system responses with woylie populations undergoing decline are consistent with the hypothesis that immunologic stressors may be involved. Preliminary results that indicate the prevalence of gastrointestinal and blood parasites in woylies from the Upper Warren were significantly higher than those from Karakamia (Parker et al., 2008) further support this hypothesis.

Differences between sexes in the RBC count are consistent with other marsupials: an increased erythrocyte concentration was found in male allied rock-wallabies, *Petro-*

TABLE 4. Sample size (*n*), mean, standard deviation (SD), and 5th and 95th percentiles of hematologic parameters, by gender, in woylie (*Bettongia penicillata ogilbyi*) populations.[a]

	RBC[a] ($\times 10^{12}$/L)	HGB[a] (g/L)	HCT (L/L)	MCV (fL)	MCH (pg)	CHCM (g/L)
Perup males						
n	64	64	64	64	64	64
Mean (SD)	11.78 (1.05)	162.41 (12.2)	0.53 (0.05)	45.05 (2.71)	13.83 (0.97)	302.61 (16.14)
5th–95th Perc[c]	9.88–13.6	135.75–181	0.43–0.61	40.73–50.18	12.45–15.3	270.5–329.25
Perup females						
n	40	40	40	40	40	39
Mean (SD)	11.46 (1.14)	159.75 (13.4)	0.52 (0.04)	45.84 (3.52)	13.99 (0.91)	302.82 (16.45)
5th–95th Perc	9.38–13.15	138.05–184.75	0.45–0.6	40.13–53.26	12.41–15.7	265–322
Kingston males						
n	29	29	29	29	29	29
Mean (SD)	10.43 (1.11)	151.93 (16.69)	0.5 (0.05)	47.98 (3.1)	14.64 (1.52)	300.55 (14.35)
5th–95th Perc	8.62–12.97	120–181	0.43–0.59	41.45–53.45	11.1–16.95	272.5–327
Kingston females						
n	14	14	14	14	14	14
Mean (SD)	9.56 (0.62)	146.29 (14.9)	0.47 (0.04)	48.81 (4.1)	15.34 (1.57)	306.57 (11.06)
5th–95th Perc	8.44–10.55	114–168	0.39–0.54	39.8–55.1	11.6–17.5	288–326
Venus Bay CP males						
n	19	19	19	19	19	19
Mean (SD)	11.27 (1.08)	170 (13.67)	0.54 (0.04)	48.39 (3.6)	15.15 (1.04)	330.95 (12.25)
5th–95th Perc.	8.31–12.79	130–193	0.41–0.6	42.9–54.9	13.5–17.2	308–350
Venus Bay CP females						
n	13	13	13	13	13	13
Mean (SD)	11.33 (1.02)	173.69 (12.62)	0.55 (0.05)	48.32 (3.27)	15.38 (0.86)	338 (18.3)
5th–95th Perc	9.16–12.46	145–190	0.44–0.63	43.3–55.1	14–16.8	301–371
St. Peter Island males						
n	23	23	23	23	23	23
Mean (SD)	9.09 (0.99)	133.26 (19.51)	0.42 (0.05)	46.85 (4.22)	14.75 (2.25)	306.57 (14.33)
St. Peter Island females						
n	19	19	19	19	19	19
Mean (SD)	8.35 (1.7)	123.95 (27.99)	0.4 (0.08)	48.14 (3.31)	14.98 (2.28)	306.16 (14.75)

[a] RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; MCV = mean cell volume; MCH = mean corpuscular hemoglobin; CHCM = corpuscular hemoglobin concentration mean; WBC = total white blood cells; Neutro = neutrophils; Lympho = lymphocytes; Mono = monocytes; Baso = basophils; TP = total protein concentration; FBG = fibrinogen concentration; Perc = percentiles.
Perc = percentiles.

[b] (—) = FBG not established for Venus Bay Conservation Park (CP) and St. Peter Island.

gale assimilis (Spencer and Speare, 1992), greater gliders, *Petauroides volans* (Viggers and Lindenmayer, 2001), common and mountain brushtail possums, *Trichosurus vulpecula* and *Trichosurus caninum* (Presidente and Correa, 1981; Viggers and Lindenmayer, 1996), and tammar wallabies, *Macropus eugenii* in winter (McKenzie et al., 2002). In contrast to this study, no differences were reported in leukocyte parameters between genders in previous marsupial hematologic studies (Presidente and Correa, 1981; Spencer and Speare, 1992; Viggers and Lindenmayer, 1996; McKenzie et al., 2002). The sex differences between woylie could be related to different hormonal profiles and behavioral characteristics as well as to an increased suscep-

TABLE 4. Extended.

WBC ($\times 10^9$/L)	Neutro ($\times 10^9$/L)	Lympho ($\times 10^9$/L)	Mono ($\times 10^9$/L)	Eos ($\times 10^9$/L)	Baso ($\times 10^9$/L)	TP (g/L)	FBG[b] (g/L)
64	64	64	64	62	64	60	14
5.84 (2.11)	2.19 (0.99)	3.06 (1.61)	0.14 (0.12)	0.2 (0.24)	0.02 (0.03)	66.18 (3.76)	2.14 (0.54)
2.7–10.35	0.85–3.98	0.85–6.24	0–0.45	0–0.88	0–0.09	59–72	1–3
40	40	40	40	39	40	38	7
5.35 (1.81)	2.03 (0.87)	2.92 (1.82)	0.12 (0.1)	0.12 (0.13)	0.02 (0.03)	67.63 (4.28)	2.29 (0.76)
2.53–8.09	0.62–3.63	0.54–6.37	0–0.34	0–0.4	0–0.09	59.95–75.1	1–3
29	29	29	29	29	29	28	10
5.77 (2.28)	2.02 (1.1)	2.97 (1.39)	0.17 (0.15)	0.39 (0.53)	0.03 (0.05)	65.29 (5.79)	2.2 (0.42)
2.2–10.05	0.4–4.16	0.75–5.23	0–0.5	0–1.85	0–0.16	55.8–79.55	2–3
14	14	14	14	14	14	14	5
4.11 (1.08)	1.54 (0.7)	2.11 (0.77)	0.08 (0.11)	0.22 (0.16)	0.01 (0.03)	64.36 (4.48)	1.8 (0.837)
2.7–6.2	0.44–3.31	0.9–3.5	0–0.37	0–0.56	0–0.09	56–72	1–3
19	19	19	19	19	19	19	—
5.85 (2.14)	3.91 (2.2)	1.53 (1.31)	0.17 (0.11)	0.04 (0.05)	0.03 (0.04)	71.32 (10.1)	—
1.9–10.1	1.14–8.89	0.34–5.51	0.03–0.34	0–0.15	0–0.15	45–88	—
13	13	13	13	13	13	13	—
5.17 (2.52)	2.16 (1.52)	2.55 (1.51)	0.17 (0.16)	0.16 (0.15)	0.03 (0.06)	72.92 (7.09)	—
2.5–11.7	0.82–6.26	0.98–5.66	0.04–0.53	0.03–0.48	0–0.23	58–86	—
23	23	23	23	23	23	23	—
4.35 (2.63)	2.79 (1.59)	1.36 (1.31)	0.14 (0.17)	0.05 (0.07)	0.17 (0.32)	67.09 (4.06)	—
19	19	19	19	19	19	19	—
3.69 (1.87)	2.39 (1.63)	1.11 (0.78)	0.11 (0.11)	0.05 (0.06)	0.24 (0.51)	64.95 (2.99)	—

tibility of one sex to specific pathogens. This could be particularly true for eosinophil concentrations and could explain the trend of higher eosinophil concentrations in males. Males of various mammalian species are more prone to heavier parasite infestations (Wilson et al., 2002), and increased eosinophil counts is a common hematologic response in these situations (Kerr, 2002).

Climatic influence

A robust understanding of the effect of season on hematologic parameters of marsupials, particularly macropods, is restricted by the small number of published studies, small sample sizes within seasons, no or few repeated surveys over several continuous years, and variation in the climatic regions in which the studies were conducted. Our results are not immune to these issues. Nevertheless, changes in the erythron panel for woylies were consistent with the seasonal hematologic changes in tammar wallabies (McKenzie et al., 2002), western grey kangaroos, *Macropus fuliginosus* (Algar et al., 1988), agile wallabies, *Macropus agilis* (Stirrat, 2003), and mountain brush-

TABLE 5. Relationships of rainfall, period of the year (colder months versus warmer months), and population, with hematologic parameters, for the woylie (*Bettongia penicillata ogilbyi*). Each line reports standardized correlation coefficient and, between brackets, R^2 change for the variable retained in the hierarchical multiple regression models. Variables not retained in the model are indicated with dashes (—). When only one variable was retained, Spearman's rho correlation coefficient is reported.

Parameter[a]	*n*	Rainfall	Year period[b]	Population[c]	*P*-value[d]
RBC	171	0.157 (0.043)	—	−0.379 (0.141)	<0.0005
HGB	171	0.222 (0.059)	—	−0.161 (0.025)	0.001
HCT	171	0.143 (0.056)	−0.186 (0.026)	—	0.001
TP	165	—	−0.145	—	0.025
WBC	171	−0.197	—	—	0.005
Neutrophils	171	—	−0.163	—	0.012
Lymphocytes	171	−0.229	—	—	0.001
Monocytes[e]	171	—	—	—	—
Eosinophils[e]	171	—	0.139 (0.027)	0.155 (0.023)	0.008

[a] RBC = red blood cells; HGB = hemoglobin concentration; HCT = hematocrit; TP = total protein concentration; WBC = total white blood cells.

[b] Codified as "0" for colder months (March to August) and "1" for warmer months (September to February).

[c] Codified as "1" for Perup and "2" for Kingston.

[d] Statistical significance of the final model (analysis of variance) or Spearman's rho correlation.

[e] The square roots of raw variables were used in the analysis.

tail possums (Barnett et al., 1979). Similar changes in HGB and HCT in quokkas, *Setonix brachyurus*, RBC and HGB in euros, *Macropus robustus*, and TP in agile wallabies (Stirrat, 2003) were also reported and it was inferred that nutrition was responsible for these seasonal fluctuations (Barker, 1961; Ealey and Main, 1967; Shield, 1971). Rain influences the nutrient content of plants (Ealey and Main, 1967); increased nutrient content in plants, especially proteins, was positively correlated with increased erythron values in euros (Ealey and Main, 1967). Seasonal changes in diet and food quantity and quality may also explain seasonal hematologic changes in the Upper Warren populations: fungi, the main diet of woylies, are more prevalent in winter and autumn than in spring and summer (Zosky, 2012). If this hypothesis is correct, it is not surprising that once the effect of the rain was partialled out, the period of the year did not significantly influence the prediction of the majority of the erythron values. Nevertheless, no quantitative assessment of the relationship between the nutritional composition of diets and hematologic profiles has been carried out in the woylie, and similar fluctuations have been proven not to be associated with nutrition in the western grey kangaroo (Algar et al., 1988). The significant influence of the variable "population" on RBC and HGB also indicates that other factors

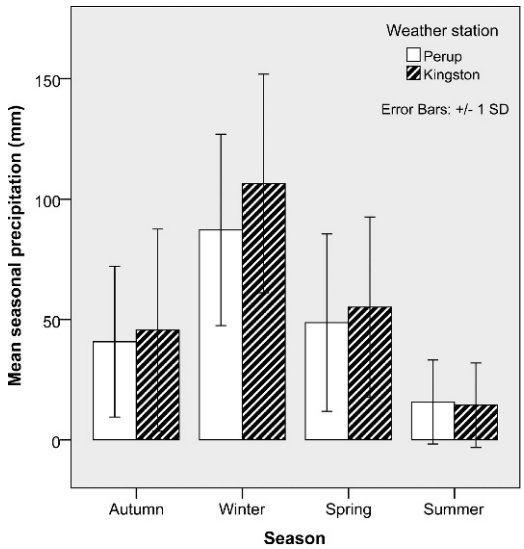

FIGURE 3. Mean seasonal precipitation (mm) for two weather stations (9616 = Perup; 9906 = Kingston) in the Upper Warren region, Western Australia.

influence these parameters, which are not simply explained by rainfall and period of the year alone. For example, nematode infestations in macropods resulted in anemia (Arundel et al., 1977), and hemo- or endoparasitism could result in seasonal fluctuation in RBC indices corresponding with seasonal changes in parasite load.

The interpretation of leukocyte variation in association with rainfall and the period of the year presented a challenge. It is likely that multiple factors are responsible for these hematologic changes and a direct, simple explanation is unlikely.

Similar patterns to those found in this study were identified in adult tammar wallabies; nevertheless, none of these differences were statistically significant (McKenzie et al., 2002)—a likely function of small sample sizes. In mountain brushtail possums and agile wallabies, parasitism was suggested as a possible explanation of seasonal variation in the concentration of eosinophils (Viggers and Lindenmayer, 1996; Stirrat, 2003). Increased exposure to parasites and vectors, or migration of larvae during the warmer months, could similarly explain the change in eosinophil counts found in woylies.

Stress associated with extreme temperatures was suggested as a possible mechanism responsible for changes in the concentration of neutrophil and lymphocytes in the brushtail possum (Baker and Gemmell, 1999). Seasonal fluctuations of WBC could support this hypothesis (lower lymphocyte and higher neutrophil counts in winter). However, the fact that neutrophil counts varied significantly with the period of the year, whereas lymphocytes did not, argues against a simple stress-related response.

Implication for conservation, management, and further studies

We provide important baseline data to evaluate woylie health and a valid basis of comparison for other hematologic investigations in macropods. The established reference ranges will facilitate the interpretation of results in ongoing and future disease investigations in this species. With due consideration of the limitations of such studies in wild animals (e.g., lack of individual's clinical history; unpredictable environmental variability), our sample size is among the largest ever used to establish hematologic reference ranges in macropods and, more generally, in marsupials (Melrose et al., 1987; Haynes and Skidmore, 1991; Spencer and Speare, 1992; Svensson et al., 1998; McKenzie et al., 2002; Clark, 2004; Wicks and Clark, 2005; Clark and Spencer, 2006; Bennett et al., 2007; Reiss et al., 2008; Vogelnest and Portas, 2008).

There were strong differences between populations and, therefore, we recommend the use of reference ranges calculated for the population of origin. When evaluating the health of newly established populations (e.g., translocated populations) or newly studied populations, wider ranges should be used (i.e., overall species). This study also represents a further contribution toward the understanding of associations between hematologic changes and environment variability. A clear pattern was evident in the erythron with respect to rainfall, which was consistent with other studies in marsupials.

Unprecedented fluctuations in the leukocyte panel were also found (i.e., associations between leukocytes and rainfall and period of the year). While we acknowledge the limitations of our study, we argue that the results from ours and other studies suggest that future researchers should concurrently assess how resources and parasites (endo-, ecto-, and hemoparasites) vary in relation to climate variation and, ultimately, what are the hematologic responses of the host to these factors. In western ringtail possums, *Pseudocheirus occidentalis*, increased WBC counts were associated with reduced survival (Clarke, 2011), indicating the potential importance that these fluctuations may have for individual and population health. Understanding the causes of these variations will also

improve management and possibly increase survival of wild macropod populations.

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